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# Multiple Rh Messenger RNA Isoforms Are Produced by Alternative Splicing

By Caroline Le Van Kim, Baya Chérif-Zahar, Virgine Raynal, Isabelle Mouro, Manuel Lopez, Jean-Pierre Cartron, and Yves Colin

Three Rh-related cDNAs have been isolated from a human bone marrow cDNA library and by polymerase chain reaction (PCR) amplification of human bone marrow and erythroblast mRNAs. They potentially encode a family of Rh protein isoforms that exhibit several unexpected structural properties as compared with the Rh polypeptide encoded by the cDNA clone identified previously. These modifications include several peptide deletions, the predicted alteration of Rh protein topology within the cell membrane, variations in the number and surface exposition of cysteine residues, and the generation of new C-terminal polypeptide segments

**T**HE RH BLOOD GROUP system has proved to be important in transfusion and clinical medicine because it has been involved in hemolytic conditions of both immune<sup>1</sup> and nonimmune origin.<sup>2</sup> This system was recognized as one of the most complex polymorphisms in humans, but the structural basis of blood group antigens has remained poorly understood until recently.<sup>3</sup> Human red blood cells (RBCs) can be commonly subdivided into Rh-positive and Rh-negative according to the presence or the absence of the D antigen, but the Rh serology is more complex, and several antigens like those of the C/c and E/e series have been defined.<sup>4</sup> The question as to whether these three sets of antigens are carried by a single protein containing multiple Rh epitopes<sup>5</sup> or by independent proteins encoded by closely linked genes<sup>6</sup> has been partly resolved by biochemical investigations which have demonstrated that the D, c, and E epitopes are more likely carried by at least three distinct homologous nonglycosylated integral membrane proteins of apparent Mr 30,000 to 32,000.<sup>3</sup> Moreover, the mRNA encoding one of the Rh polypeptides has been recently cloned,<sup>7,8</sup> and further studies by Southern blot hybridization have shown that the RH locus carried by the genome of RhD-positive individuals is composed of two strongly related genes, whereas one of these two genes is missing in RhD-negative donors.<sup>9</sup> These findings are consistent with a two-locus model of Rh inheritance,<sup>10</sup> and suggest that one of the two genes of the RH locus encodes

caused by frameshift mutations. The four Rh mRNAs now described correspond to different splicing isoforms transcribed from the same Rh gene, and all exist in the same cell lineage (erythroid). Moreover, PCR experiments indicated that at least three of these RNA species exist in reticulocytes from donors with different commonly expressed Rh phenotypes. Although the translated proteins have not yet been characterized, these results suggest that the two genes at the RH locus may direct the synthesis of several protein species possibly corresponding to different Rh antigenic variants.

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the RhD protein whereas the other encodes RhC/c and E/e polypeptides.<sup>9</sup>

How the two Rh genes control Rh antigens production and how many polypeptides are necessary to account for the large diversity of Rh antigens<sup>4,11</sup> is presently not known. In an attempt to address these issues, we report here the characterization of several Rh cDNA clones that are derived from alternative splicing of the gene encoding Cc and Ee antigens.

## MATERIALS AND METHODS

**Human bone marrow, erythroblast, and reticulocytes samples.** Bone marrow cells were collected from posterior iliac crests of healthy individuals donating bone marrow for allogeneic transplantation. Mononuclear cells from an approximately 50-mL bone marrow sample were separated over a Ficoll gradient (density = 1.077) as described previously.<sup>12</sup> Spleen erythroblast RNAs (a generous gift of Dr P.H. Romeo, INSERM U91, Créteil, France) from an adult  $\beta$ -thalassemic patient and human bone marrow RNAs were extracted by the guanidine isothiocyanate/cesium chloride method.<sup>13</sup> Reticulocyte RNAs were prepared from 50 mL of whole blood as described.<sup>14</sup>

**cDNA library screening.** The probe specific for the N-terminal region of the Rh polypeptides<sup>7</sup> was labeled using a random priming kit (Boehringer Mannheim, Mannheim, Germany) and used to screen a human bone marrow  $\lambda$ gt11 cDNA library (Clontech, Palo Alto, CA) as described.<sup>7</sup>

**Polymerase chain reaction (PCR) amplification of RNA.** Two sets of primers were used for PCR. -PCR 1: Primer "a" (sense primer: 5' GCA CAG AGA CGG ACA CAG 3') deduced from the 5' noncoding region of clone RhIXb<sup>7</sup> and primer "b" (anti-sense primer: 5' AGG AGA CCA GAC GTG AG 3') specific of nucleotides 1148 to 1132 (+1 taken as the first residue of the initiator AUG), which are deleted in clone RhVI. -PCR2: Primer "a" (as above) and primer "c" (anti-sense primer: 5' TCC AAC AGC CAA ATG AGG 3') deduced from the 3' end of the RhIXb coding sequence.<sup>7</sup> RNA-PCR amplifications were performed as described previously<sup>15</sup> with 0.5  $\mu$ g of RNA from human bone marrow, spleen, erythroblast, or reticulocyte as templates. cDNA products were separated on agarose gels and hybridized with the original RhIXb cDNA<sup>7</sup> or with oligonucleotide probe A ("pA", 5' TCA GGT GAC ACG AGG TA 3') specific of nucleotides 862 to 846, which are deleted in clone RhVIII.

**DNA sequencing.** Inserts from recombinant  $\lambda$ gt11 clones and PCR product (Rh4) were subcloned in PUC 18 vectors and sequenced on both strands by the dideoxy chain termination

We have Rh cDNA peptide,<sup>7</sup> v library with mon to the probe, two also detect first because the Rh locus subsequent species pot

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Submitted December 31, 1991; accepted April 27, 1992.

Supported by the Institut National de la Santé et de la Recherche Médicale (INSERM) and by the Caisse Nationale d'Assurance Maladie des Travailleurs Salariés.

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method<sup>16</sup> with a Pharmacia T7 sequencing kit. Universal primers or specific oligonucleotides were used as internal primers.

## RESULTS

We have previously reported the sequence of a 1,376-bp Rh cDNA clone (RhIXb) encoding a 417-amino acid polypeptide,<sup>7</sup> which was isolated from a human bone marrow library with a probe specific of the N-terminal region common to the RhD, c, and E polypeptides. Using this same probe, two other positive clones (RhVI and RhVIII) were also detected in this library but were not further analyzed at first because they were smaller than the RhIXb clone. As the *Rh* locus is highly polymorphic,<sup>4,11</sup> these clones were subsequently fully sequenced to characterize other mRNA species potentially encoding different Rh polypeptides.

Sequence analysis indicated that these cDNAs represent two isoforms of the RhIXb cDNA lacking different sequences of its coding region (Fig 1A). Indeed, clone RhVIII (971 bp) extends from the 5' (position -6) to the 3' (equivalent position 1,418) noncoding region of the RhIXb cDNA, but lacks nucleotides 487 to 939 (+1 taken as the first residue of the initiator AUG). Because this deletion does not alter the reading frame of the resulting mRNA, the deduced RhVIII encoded protein represents a shortened form of the RhIXb protein missing amino acids 163 to 313 (Fig 1B) and is thus composed of 266 amino acids (calculated Mr 29,200). As compared with the RhIXb protein, three of the six cysteine residues, including the exofacial Cys-285, have been lost in the RhVIII protein (Fig 1A). In addition, there are three nonconservative changes (positions 16, 60, and 68), the most noticeable being the W → C exchange at position 16. Accordingly, the predicted RhVIII protein carries only four cysteine residues.

Clone RhVI (1,085 bp), also starts in the 5' noncoding region of clone RhIXb (position -9) and extends up to a poly(A) addition site (position 1,433; not shown) that was not present in the Rh clones previously reported.<sup>7,8</sup> This clone exhibits two deletions as compared with the RhIXb coding region. The first deletion corresponds to nucleotides 487 to 801 (amino acids 163 to 267) and does not alter the reading frame, while the second deletion corresponds to nucleotides 1,074 to 1,153 (amino acids 359 to 384) and modifies the reading frame of the downstream sequence (Fig 1, A and B) with a premature stop codon after position 1,196. Therefore, the RhVI cDNA encodes a predicted 267-amino acid polypeptide (calculated Mr 28,600) that represents another shortened form of the RhIXb protein with a different and shorter C-terminal sequence (Fig 1B). Moreover, this protein contains six cysteine residues. Five correspond to cysteines in RhIXb and the sixth is in the new carboxy terminus at position 266.

A fourth Rh cDNA isoform, Rh4, which was not present in the bone marrow library, was produced during PCR experiments performed with bone marrow mRNA. As compared with the RhIXb cDNA, this clone of 1,235 bp extends from nucleotide -19 to +1,278 and exhibits a deletion in the coding sequence between nucleotides 940 and 1,073 (amino acids 314 to 358; Fig 1A), which in turn results in a

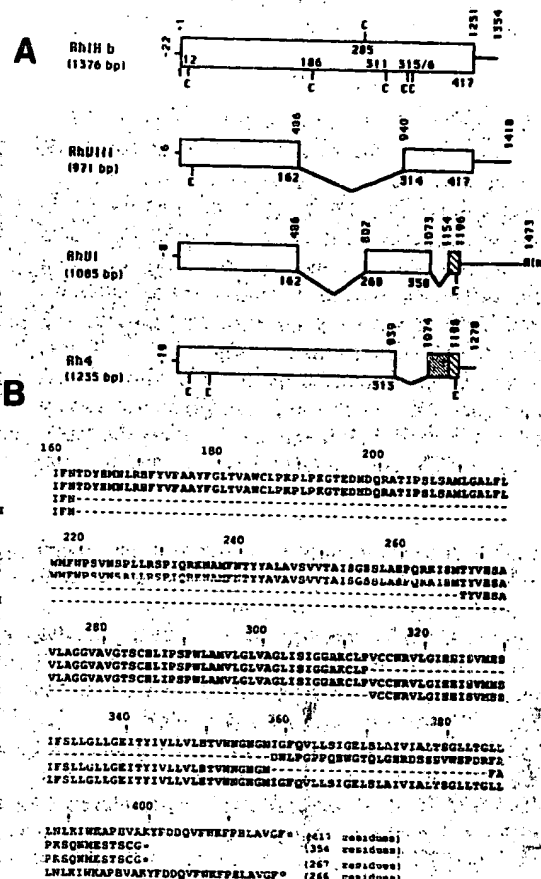


Fig 1. Schematic representation of the four Rh RNA splicing isoforms. (A) Open boxes represent coding sequences and single lines the noncoding regions. Matched boxes indicate regions of RhVI and Rh4 encoding amino acids unrelated to those encoded by RhIXb (taken as prototype mRNA). Numbers above the boxes are for nucleotide positions, +1 being taken as the first nucleotide of the translation initiation AUG of clone RhIXb. Numbers below and inside the boxes refer to amino acid positions, +1 being taken as the initiating methionine. Here, the nucleotides and amino acids have been renumbered for convenience compared with the previously described RhIXb clone.<sup>7</sup> The cysteine residues (C) indicated on RhVIII, RhVI, and Rh4 are those not present on RhIXb (see text). Sequences of the RhIXb, RhVIII, RhVI, and Rh4 clones are deposited to the GenBank database (accession numbers M34015, X63096, X63095, X63098). (B) C-terminal amino acid sequence of the Rh protein isoforms encoded by the RhIXb, Rh4, RhVI, and RhVIII cDNAs (from position 160). Dashes refer to amino acids lacking in Rh4, RhVI, and RhVIII isoforms.

translation frameshift generating a premature stop at the same position that was used in the RhVI clone (Fig 1B). The new predicted protein has 354 amino acid residues (calculated Mr 38,500) and carries seven cysteine residues, including four from RhIXb (Cys-315 and -316 have been lost) and three additional ones at positions 16 (W → C exchange), 61 (G → C exchange), and 353 (in the new C-terminal segment). It is noteworthy that both RhVI and Rh4 share the same carboxy-terminal fragment of 14 amino acids not found in the other Rh protein isoforms identified so far (Fig 1B).

Because we have isolated and partially determined the structure of the gene for the RhIXb-encoded protein (our unpublished data, December 1991), we found that the boundaries of the deletions identified in the RhVI, RhVIII, and Rh4 cDNAs, as compared with the RhIXb cDNA, all correspond to exact intron/exon transitions and that these clones lack sequences specific of exons 4, 5, and 8 (RhVI); exons 4, 5, and 6 (RhVIII), and exon 7 (Rh4). These data strongly suggest that the RhIXb, RhVIII, RhVI, and Rh4 cDNAs (entire sequences deposited to the GenBank/EMBL database, accession numbers M34015, X63096, X63095, X63098) correspond to four Rh mRNA isoforms produced from the same gene (*Cc/Ee*, see below) by alternative splicing of separate exons.

All of these cDNAs were also identified when mRNAs extracted from human thalassemic spleen erythroblasts were amplified by the PCR technique between primers specific of the 5' and 3' ends of the RhIXb cDNA (see Materials and Methods) and sequenced (not shown). These results indicate that the different Rh mRNA isoforms are present in the same cell lineage, erythroid, and do not encode Rh proteins differently expressed in various tissues. To determine whether these splicing events might represent different Rh antigenic variants, PCR amplifications were performed on RNA extracted from reticulocytes of RhD-positive individual donors with the homozygous DC-Cee, Dccee, and DccEE phenotypes (Fig 2). From the sequence of the RhIXb, RhVIII, and RhVI clones, it was expected that the various mRNA isoforms present in the reticulocyte preparations could be distinguished by the size of the PCR amplification products generated between primers "a" and "c" (Fig 2A, PCR 2). As only a limited amount of material was available, the amplification products were detected by hybridization with the RhIXb cDNA probe. Two major signals were detected, as shown from Fig 2B, lanes 2. While the upper band, migrating above 1,200 bp, clearly corresponded to the RhIXb cDNA (expected size: 1,267 bp), it could not be assessed whether the lower

band, migrating around 840 bp, was related to the RhVIII and/or RhVI mRNA (expected sizes: 813 and 870 bp, respectively). To discriminate between these possibilities, oligonucleotides specific of RhVIII (primer "b") or of RhVI (probe "pA") were used as amplimer (PCR1) or as probe, respectively (see Fig 2A). Hybridization of the RhIXb cDNA probe with a fragment amplified between primers "a" and "b" (VIII-specific) and migrating around 730 bp (Fig 2B, lanes 1) indicated the presence of the RhVIII isoform (expected size: 713 bp). The presence of the RhVI isoform was shown by hybridization of the oligonucleotide probe "pA" (RhVI-specific) with a product amplified between primers "a" and "c" (expected size: 870 bp) and migrating slightly above 840 bp (Fig 2C, lanes 2). Accordingly, probe "pA" failed to hybridize with the RhVIII fragment of 713 bp amplified between primers "a" and "b" (Fig 2C, lanes 1). A PCR product migrating slightly below 1,200 bp corresponding to the RhIXb isoform was also amplified between primers "a" and "b" (expected size: 1,167 bp) and was detected both by the RhIXb and "pA" probes (Fig 2B and C, lanes 1). Because exactly the same amplification and hybridization patterns were obtained with all the Rh-typed reticulocyte samples, the present results indicate that the Rh spliceform mRNAs may not account for the C to c and E to e allelic polymorphism but may explain the presence of the Cc and Ee antigens on different polypeptides (see Discussion). Probably because of a low expression level in reticulocytes, the Rh4 cDNA was identified during these experiments only when a primer specific of its deletion junction was used (not shown).

Hydropathy analysis and secondary structure predictions according to Engelman et al<sup>17</sup> predict that the RhIXb, RhVI, RhVIII, and Rh4 proteins contain 13, 8, 8, and 10 transmembrane domains, respectively, arranged as shown in Fig 3. Examination of these models proves interesting because of the amino acid deletion in RhVIII which links res. 162 to 314 of RhIXb, the topology of res. 162 to 314 at the C-terminus of RhVIII is expected to be in reverse

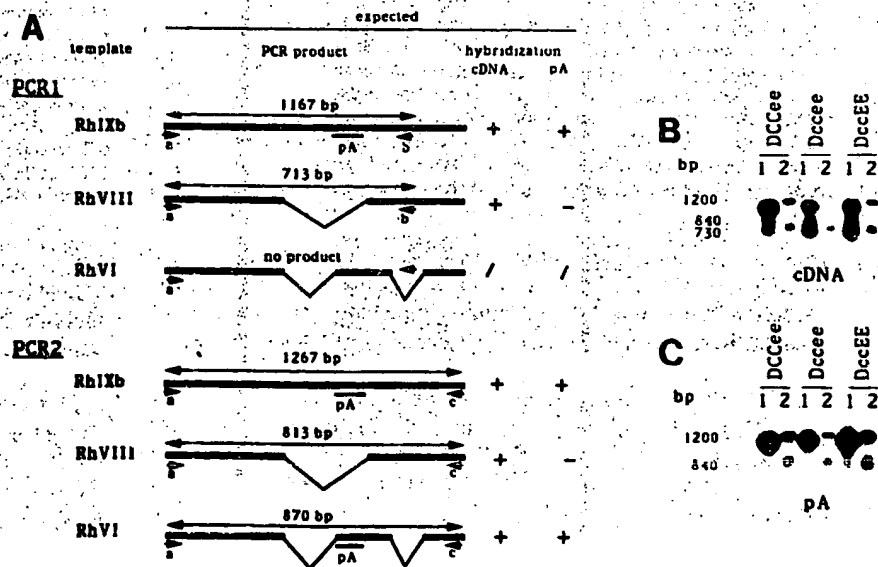


Fig 2. PCR amplification of reticulocyte Rh spliceforms RNAs. (A) Schematic diagram (not to scale) indicating the location of the amplimers used and of the oligonucleotide probe "pA" on the RhIXb, VIII, and VI cDNAs. The size and the hybridization pattern of the expected PCR products are indicated. Total reticulocyte RNAs from donors with the indicated Rh phenotypes were subjected to PCR amplification between primers "a" and "b" (PCR1) or "a" and "c" (PCR2). cDNAs products were resolved on agarose gel (the sizes of DNA markers are indicated) and characterized by hybridization with the RhIXb cDNA (B) or with the "pA" oligonucleotide probe (C).

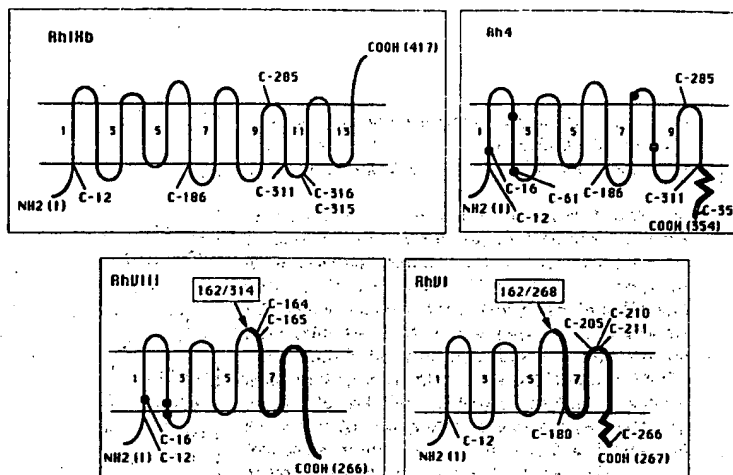
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Fig 3. Predicted membrane topology of the Rh protein isoforms. Topologic models of the putative proteins encoded by RhVI, RhVIII, and Rh4 were predicted from hydropathy analysis according to Engelman et al<sup>17</sup> using a 20-amino acid window and compared with the prototype RhIXb protein.<sup>7</sup> Only the uneven transmembrane segments are numbered. Position of the cysteine residues (C) on each polypeptide chains are also given. Heavy lines in RhVIII and RhVI refer to identical sequences with a reverse orientation in the prototype RhIXb protein that are generated by connecting residues 162 to 314 (RhVIII) and 162 to 268 (RhVI) of RhIXb (see text). The wavy line refers to new protein sequences generated by frameshift mutations in RhVI and Rh4. Amino acid polymorphisms are indicated by black circles.



orientation compared to their predicted position in RhIXb (res. 315 to 417). Accordingly, the carboxy end of RhVIII is predicted to be intracellular and the Cys-164 and Cys-165 are predicted to be exofacial, whereas their position in RhIXb (Cys-315 and Cys-316) is intracellular. (2) In RhVI the protein segment 163 to 253 is in a reverse orientation as compared with its position in RhIXb (res. 268 to 358). Therefore, the cysteines 285, 311, 315, and 316 of RhIXb are also reversely oriented as Cys-180, -205, -210, and -211, respectively, in RhVI. In addition, the Cys-266 present on the new carboxy terminus of RhVI is intracellular. (3) The Rh4 protein is deleted of amino acids 314 to 358 of RhIXb and therefore both proteins have a similar topology up to position 313, including the surface exposition of Cys-12, -186, -285 (extracellular), and -311. Cys-16 is located within the first transmembranous domain, whereas Cys-61 lies at the cytoplasmic side. The carboxy terminal residues 314 to 354 of Rh4, including the Cys-353, are generated by frameshift translation and are located on the cytoplasmic side of the membrane.

#### DISCUSSION

Four Rh-related cDNAs (RhIXb, RhVI, RhVIII, and Rh4) have been characterized by sequence analysis of cloned and/or PCR-amplified human bone marrow and erythroblast mRNAs. Because of their identical representation in the cDNA libraries, we assume that equivalent amounts of the RhIXb-, RhVI-, and RhVIII-specific mRNAs are present in erythroid tissues and presumably identically translated. However, the Rh4 mRNA isoform might be less abundantly expressed because it was detected only after PCR amplification.

By Southern analysis of the DNA from individuals of different Rh phenotypes (combinations of D, C, c, E, and e antigens) we have shown recently that the RH locus is composed of two related genes and that one of these genes should encode both the C/c and E/e proteins while the other, which is absent from RhD-negative individuals, should encode the D polypeptide.<sup>9</sup> In preliminary investigations on the comparative structure of these two genes we have cloned genomic restriction fragments specific of the

such defined D and CcEe genes. Sequence analysis showed several polymorphisms between the two genes (our unpublished results, December 1991) and indicated that the RhIXb mRNA is transcribed from the Cc/Ee gene, which is in agreement with partial RhD protein sequence analysis suggesting that the RhIXb cDNA does not encode the D polypeptide.<sup>8</sup> Interestingly, comparison of cDNAs and genomic structures also indicates that the RhVIII, RhVI, and Rh4 mRNAs are transcribed from the same gene as RhIXb and correspond to various splicing isoforms lacking different exons.

While most examples of alternate mRNA splicing are associated with different tissue-specific or developmentally regulated gene expression,<sup>18</sup> the various Rh mRNAs identified so far are expressed within a single tissue, human erythroid cells, potentially giving rise to a family of Rh polypeptide isoforms. Moreover, PCR experiments indicate that at least three of these spliceforms are cotranscribed in reticulocytes from individual donors expressing different homozygous combination of the Cc/Ee antigens. Therefore, it is assumed that alternative splicing events may account at least for the synthesis of the three already characterized homologous Rh polypeptides<sup>19</sup> by the two genes at the RH locus, and the present results suggest that more than three Rh proteins are probably present in human erythrocytes. Which Rh antigens, if any, are carried by the different Rh protein isoforms may not be resolved easily because, in preliminary experiments, eukaryotic cells transfected with the recombinant Rh cDNAs failed to react with any of the D, C, c, E, and e Rh antibodies (our unpublished data, December 1991), possibly because the blood group antigenicity of the Rh proteins may require a specific conformation occurring only in erythrocyte membranes.<sup>3,20</sup> However, from immunologic studies performed on different Rh typed RBCs with polyclonal antibodies raised against synthetic peptides specific for the RhIXb-encoded polypeptide, it is more likely that this mRNA encodes the E or e antigens (our unpublished results, December 1991). Accordingly, the splicing isoforms RhVI, RhVIII, and Rh4 might account for the expression of the C or c antigens by the Cc/Ee gene, because all of these predicted proteins

ification of spliceforms. The diagram shows the locations used and the probe used. RhVIII, and VI the hybridization expected indicated. To is from do-d Rh phenotypes PCR on primers or "a" and products were (the sizes indicated) / hybridization DNA (B) or nucleotide

differ from RhIXb in their C-terminal region by drastic structural changes such as: (1) the appearance of new amino acid sequence(s) at the limits of deleted region(s); (2) the reverse orientation of the C-terminal peptide region (RhVI and RhVIII), as predicted from hydropathy plot analysis and the concomitant extracellular exposition of amino acids and cysteine residues that are intracellularly exposed on RhIXb (Fig 3); (3) the intracellular exposition of C-terminal radioiodinable tyrosine residues that were extracellularly exposed on RhIXb; (4) the loss of the unique exofacial Cys-285 (RhVI and RhVIII), which might be critical for antigenic expression<sup>21,23</sup>; (5) the new C-terminal amino acid sequence (RhVI and Rh4) resulting from frameshift translation, although this peptide should not

participate directly in new surface antigens because it is predicted as intracytoplasmic.

Obviously, the new putative Rh protein structures described in this report may well explain the large number of antigenic specificities associated with Cc and Ee polymorphisms, including those of the more unusual compound antigens like Cc, cE, ce, and CE.<sup>4</sup> We conclude that the extreme polymorphism of the Rh system at the antigenic level is associated with a great heterogeneity at the mRNA level. Characterization of these multiple mRNA structures and of their deduced encoded proteins may provide a molecular basis to RH locus expression under varying genetic conditions.

## REFERENCES

1. Mollison PL, Engelfriet CP, Contreras M: Blood Transfusion in Clinical Medicine (ed 8). Oxford, UK, Blackwell, 1987
2. Nash R, Shojania AM: Hematological aspect of Rh deficiency syndrome: A case report and review of the literature. *Am J Hematol* 24:267, 1987
3. Agre P, Cartron JP: Molecular biology of the Rh antigens. *Blood* 78:551, 1991
4. Race RR, Sanger R: Blood Groups in Man (ed 6). Oxford, UK, Blackwell, 1975
5. Wiener AS: The Rh series of allelic genes. *Science* 100:595, 1944
6. Race RR: An "incomplete" antibody in human serum. *Nature* 153:771, 1944
7. Chérif-Zahar B, Bloy C, Le Van Kim C, Blanchard D, Bailly P, Herman J P, Salmon C, Cartron JP, Colin Y: Molecular cloning and protein structure of a human blood group Rh polypeptide. *Proc Natl Acad Sci USA* 87:6243, 1990
8. Avent ND, Ridgwell K, Tanner MJA, Anstee DJ: cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (Rhesus)-blood-group-antigen expression. *Biochem J* 271:821, 1990
9. Colin Y, Chérif-Zahar B, Le Van Kim C, Raynal V, Van Huffel V, Cartron JP: Genetic basis of the RhD-positive and RhD-negative blood group polymorphism as determined by Southern analysis. *Blood* 78:2747, 1991
10. Tippett P: A speculative model for the Rh blood groups. *Ann Hum Genet* 50:241, 1986
11. Issitt PD: The Rh blood group system, 1988: Eight new antigens in nine years and some observations on the biochemistry and genetics of the system. *Transfus Med Rev* 3:1, 1989
12. Lopez M, Andreu G, Baujean F, Ehrsam A, Gerota J, Hervé P: Human bone marrow processing in view of further in vitro treatment and cryopreservation. *Rev Fr Transfus Immunohématol* 28:411, 1985
13. Davis LG, Dibner MD, Battey JF: Guanidine isothiocyanate preparation of total RNA. in *Basic Methods in Molecular Biology*. New York, NY, Elsevier Science, 1986, p 131
14. Goossens M, Kan YY: DNA analysis in the diagnosis of hemoglobin disorders. *Methods Enzymol* 76:805, 1981
15. Colin Y, Le Van Kim C, Tsapis A, Clerget M, d' Aurio L, London J, Galibert F, Cartron JP: Human glycophorin C. Gene structure and rearrangement in genetic variants. *J Biol Chem* 264:3773, 1989
16. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463, 1977
17. Engelman D, Steitz T, Goldman A: Identifying nonpolar transbilayer helices in amino acid sequences of membrane proteins. *Annu Rev Biophys Chem* 15:321, 1986
18. Smith CWJ, Patton JG, Nadal-Ginard B: Alternative splicing in the control of gene expression. *Annu Rev Genet* 23:527, 1989
19. Bloy C, Blanchard D, Dahr W, Beyreuther K, Salmon C, Cartron JP: Determination of the N-terminal sequence of human red cell Rh(D) polypeptide and demonstration that the Rh(D), (c) and (E) antigens are carried by distinct polypeptide chains. *Blood* 72:661, 1988
20. Cartron JP: Recent advances in the biochemistry of blood-group Rh antigens. in Rouger P, Salmon C (eds): *Monoclonal Antibodies Against Human Red Blood Cell and Related Antigens*. Paris, France, Arnette, 1987, p 69
21. Green FA: Erythrocyte membrane sulfhydryl groups and Rh antigen activity. *Immunochemistry* 4:247, 1967
22. Green FA: The mode of attenuation of erythrocyte membrane Rho(D) antigen activity by 5,5'-dithiobis-(2-nitrobenzoic acid) and protection against loss of activity by bound anti-Rho(D) antibody. *Mol Immunol* 20:769, 1983
23. Ridgwell K, Roberts SJ, Tanner MJA, Anstee DJ: Absence of two membrane proteins containing extracellular thiol groups in Rh-null human erythrocytes. *Biochem J* 213:267, 1983